Form PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (Rev. 1-98)		Attorney's Docket Number 02811-0141US
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. Application No. (if known, see 37 CFR 1.5) 09/857333
International Application No. PCT/CA99/01156	International Filing Date December 3, 1999	Priority Date Claimed December 4, 1998

Title of Invention

METHOD FOR THE TREATMENT OF INFLAMMATION

Applicant(s) for DO/EO/US

Nigel C. Phillips
Mario C. Filion

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information: Return Postcard

Express Mail Label No. EL610731006US	Date: June 4, 2001	Page 1 of 2

U.S. Application No. (if known, see 37 CFR 1.5) 09/857333	International Application No. PCT/CA99/01156	Attorney's Docket Number 02811-0141US

17. ☒ The following fees are submitted: CALCULATIONS PTO USE ONLY

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.... $970.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO..... $840.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $760.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) $670.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) $96.00

ENTER APPROPRIATE BASIC FEE AMOUNT =				$840.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$130.00	
Claims	Number Filed	Number Extra	Rate		
Total claims	8 - 20 =	0	x 18.00	$	
Independent Claims	3 - 3 =	0	x 78.00	$	
Multiple Dependent Claims (if applicable)			+ 260.00	$	
TOTAL OF ABOVE CALCULATIONS =				$970.00	
Reduction of 1/2 for filing by small entity, if applicable. Applicant claims small entity status. -				$485.00	
SUBTOTAL =				$485.00	
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
TOTAL NATIONAL FEE =				$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$	
TOTAL FEES ENCLOSED =				$485.00	
				Amount to be refunded:	$
				charged:	$

a. ☒ A check in the amount of $485.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 11-0855 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. 11-0855. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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Patents

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:		
PHILLIPS, NIGEL C. **FILION, MARIO C.**	Examiner:	**Not Yet Assigned**
	Art Unit:	**Not Yet Assigned**
Serial No.: **Filed Concurrently Herewith, U. S. National Phase of PCT/CA99/01156 filed December 3, 1999**		
Filed: **June 4, 2001**		
For: **METHOD FOR THE TREATMENT OF INFLAMMATION**		

PRELIMINARY AMENDMENT

Box PCT Attn: DO/EO/US
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the present application, please enter the following Amendments and consider the appended remarks.

In the Specification:

On page 1, line 3, after the word "claims" please insert --priority to PCT/CA99/01156 filed December 3, 1999 which claims--

Express Mail No. EL610731006US
June 4, 2001.

In the Claims:

Prior to examination of the above-referenced patent application, please cancel Claims 3-10 and add the following claims:

11. (New) The method of Claim 1, wherein the effective amount is effective to induce the synthesis of cytokine IL-10.

12. (New) The method of Claim 2, wherein the effective amount is effective to induce the synthesis of cytokine IL-10.

13. (New) The method of Claim 1, wherein the pharmaceutically acceptable carrier is selected from the group consisting of a liquid carrier and a solid carrier.

14. (New) The method of Claim 2, wherein the pharmaceutically acceptable carrier is selected from the group consisting of a liquid carrier and a solid carrier.

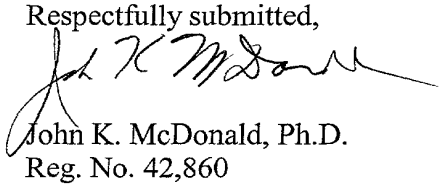
15. (New) A composition comprising *Mycobacterium phlei*-DNA preserved and complexed on a *Mycobacterium phlei* cell wall (MCC) and a pharmaceutically acceptable carrier.

16. (New) Use of the composition of Claim 15 for treating or preventing inflammation in an animal.

By this amendment, Claims 3-10 are cancelled and Claims 11-16 are added. There are now eight (8) claims pending in this case. These are 1, 2 and 11-16. For the Examiner's convenience, a complete set of pending claims is attached.

No additional fees are believed due; however, the Commissioner is hereby authorized to charge any deficiency, or credit any overpayment, to Deposit Account No. 11-0855.

Respectfully submitted,



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Attorney Docket No. 42368-258914 (02811-0141US)

Pending Claims

1. A method of preventing an inflammation in an animal, comprising administering to the animal an effective amount of a composition comprising *Mycobacterium phlei*-DNA preserved and complexed on a *Mycobacterium phlei* cell wall (MCC) and a pharmaceutically acceptable carrier, thereby preventing the inflammation in the animal.

2. A method of treating an inflammation in an animal, comprising administering to the animal an effective amount of a composition comprising *Mycobacterium phlei*-DNA preserved and complexed on a *Mycobacterium phlei* cell wall (MCC) and a pharmaceutically acceptable carrier, thereby treating the inflammation in the animal.

11. (New) The method of Claim 1, wherein the effective amount is effective to induce the synthesis of cytokine IL-10.

12. (New) The method of Claim 2, wherein the effective amount is effective to induce the synthesis of cytokine IL-10.

13. (New) The method of Claim 1, wherein the pharmaceutically acceptable carrier is selected from the group consisting of a liquid carrier and a solid carrier.

14. (New) The method of Claim 2, wherein the pharmaceutically acceptable carrier is selected from the group consisting of a liquid carrier and a solid carrier.

15. (New) A composition comprising *Mycobacterium phlei*-DNA preserved and complexed on a *Mycobacterium phlei* cell wall (MCC) and a pharmaceutically acceptable carrier.

16. (New) Use of the composition of Claim 15 for treating or preventing inflammation in an animal.

Title: METHOD FOR THE TREATMENT OF INFLAMMATION

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Serial No. 60/110,943 filed December 4, 1998.

FIELD OF INVENTION

The present invention relates to a mycobacterial deoxyribonucleic acid (B-DNA) preserved and complexed on a mycobacterial cell wall (BCC), wherein the BCC is effective for treating an inflammation in an animal. More particularly, the present invention relates to *Mycobacterium phlei (M. phlei)* deoxyribonucleic acid (M-DNA) preserved and complexed on M. phlei cell wall (MCC), wherein the MCC is effective for treating an inflammation in an animal.

BACKGROUND OF THE INVENTION

Inflammation is a complex process initiated by tissue damage. Although inflammation has evolved as a protective response against injury and infection, in certain cases such as, but not limited to, immune-mediated inflammation, osteoarthritis, rheumatoid arthritis, glomerulonephritis, cystitis and colitis inflammation itself is the problem. In these cases, the inflammatory response continues and can be only temporarily modified by the administration of anti-inflammatory agents such as aspirin, nonsteroidal anti-inflammatory drugs and cortisone. These drugs act on the metabolic pathways involved in the elaboration and activation of the pharmacological mediators of inflammation. However, these anti-inflammatory agents have numerous undesirable side effects, and cannot be tolerated by certain individuals.

Therefore, there is a continuing need for novel therapeutic agents that reduce inflammation without having deleterious side effects. Moreover, such therapeutic agents should be simple and relatively inexpensive to prepare, their activity should be reproducible among preparations, their activity should remain stable over time,

and their anti-inflammatory effects should be achievable with dose regimens that are associated with minimal toxicity.

SUMMARY OF THE INVENTION

The present invention satisfies the above need by providing a mycobacterial deoxyribonucleic acid (B-DNA) preserved and complexed on a mycobacterial cell wall (MCC), wherein the BCC is effective in treating an inflammation in an animal having an inflammation. More particularly, the present invention provides a *Mycobacterium phlei (M. phlei)* deoxyribonucleic acid (M-DNA) preserved and complexed on *M. phlei* cell wall (MCC), wherein the MCC is effective in treating an inflammation in an animal having an inflammation.

MCC is simple and relatively inexpensive to prepare, its activity is reproducible among preparations, it remains therapeutically stable over time, and it is effective at dose regimens that are associated with minimal side-effects even upon repeated administration.

To prepare MCC, *M. phlei* are grown in liquid medium and harvested. The *M. phlei* are disrupted, and the solid components of the disrupted *M. phlei* are collected by centrifugal sedimentation. The solid components are deproteinized, delipidated, and washed. DNase-free reagents are used to minimize M-DNA degradation during preparation.

A composition comprising MCC and a pharmaceutically acceptable carrier is administered in an amount effective to prevent, reduce and eliminate an inflammation in an animal, including a human. The unexpected and surprising ability of MCC to reduce inflammation, while itself having minimal side-effects, addresses a long felt unfulfilled need in the medical arts and provides an important benefit for animals, including humans.

Accordingly it is an object of the present invention to provide a composition and method effective to treat an inflammation in an animal having an inflammation.

Another object of the present invention is to provide a

composition and method effective to reduce an inflammation in an animal having an inflammation.

Another object of the present invention is to provide a composition and method effective to prevent an inflammation in an animal.

Another object of the present invention is to provide a composition and method effective to eliminate an inflammation in an animal having an inflammation.

Another object of the present invention is to provide a composition and method effective to stimulate IL-10 synthesis in an animal.

Another object of the present invention is to provide a composition and method effective to reduce immune-mediated inflammation in an animal having an immune-mediated inflammation.

Another object of the present invention is to provide a composition and method effective to reduce the inflammation of osteoarthritis in an animal having osteoarthritis.

Another object of the present invention is to provide a composition and method effective to reduce the inflammation of rheumatoid arthritis in an animal having rheumatoid arthritis.

Another object of the present invention is to provide a composition and method effective to reduce the inflammation of glomerulonephritis in an animal having glomerulonephritis.

Another object of the present invention is to provide a composition and method effective to reduce the inflammation of colitis in an animal having colitis.

Another object of the present invention is to provide a composition and method effective to reduce the inflammation of cystitis in an animal having cystitis.

Another object of the present invention is to provide a composition that can be prepared in large amounts.

Another object of the present invention is to provide a

composition that is relatively inexpensive to prepare.

Another object of the present invention is to provide a composition that remains stable over time.

Another object of the present invention is to provide a composition that maintains its effectiveness over time.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiment and the appended claims.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1. Effect of intravenous, intraperitoneal, oral and subcutaneous MCC on mouse foot pad volumes at 0, 24, 48, 72 and 96 h after carrageenan injection. Results are the mean ± SD (vertical line) for 8 mice per group.

FIG. 2. Effect of intravenous, intraperitoneal, oral and subcutaneous MCC on mouse footpad volume at 48 h after carrageenan injection. Results are the mean ± SD (vertical line) for 8 mice per group.

FIG. 3. Effect of intraperitoneal MCC on TNF-alpha and IL-10 synthesis at 0, 3, 6 and 24 h after administration. Results are the mean ± SD (vertical line) for 4 mice per group.

FIG. 4. Effect of intraperitoneal, intravenous and oral MCC on IL-10 synthesis at 6 h after administration. Results are the mean ± SD (vertical line) for 4 mice per group.

DETAILED DESCRIPTION OF THE INVENTION

The present invention comprises a mycobacterial DNA (B-DNA) preserved and complexed on a mycobacterial cell wall (BCC), wherein the BCC is effective in treating an inflammation in an animal having an inflammation. More particularly, the present invention comprises *M. phlei* DNA (M-DNA) preserved and complexed on *M. phlei* cell wall (MCC), wherein the MCC is effective in treating an inflammation in an animal having an inflammation. The present invention further comprises a method for preventing an

inflammation in an animal and for eliminating an inflammation in an animal having an inflammation.

As used herein, "treat" relates to a reduction in the volume, pain or spread of an inflammation.

Methods to increase the anti-inflammatory activity of MCC include, but are not limited to, chemically supplementing or biotechnologically amplifying stimulatory sequences or conformations of the M-DNA preserved and complexed on the *M. phlei* cell wall (MCC) and complexing the MCC to natural or synthetic carriers.

MCC is administered in a pharmaceutically acceptable carrier including, but not limited to, a liquid carrier and a solid carrier. Liquid carriers are aqueous carriers, non-aqueous carriers or both and include, but are not limited to, aqueous suspensions, oil emulsions, water in oil emulsions, water-in-oil-in-water emulsions, site-specific emulsions, long-residence emulsions, sticky-emulsions, microemulsions, nanoemulsions and liposomes. Solid carriers are biological carriers, chemical carriers or both and include, but are not limited to, microparticles, nanoparticles, microspheres, nanospheres, minipumps, bacterial cell wall extracts and biodegradable or non-biodegradable natural or synthetic polymers that allow for sustained release of the MCC. Such polymers can be implanted in the vicinity of where delivery is required. Polymers and their use are described in, for example, Brem et al., J. Neurosurg. 74: 441-446 (1991).

Preferred aqueous carriers include, but are not limited to, DNase-free water, DNase-free saline and DNase-free physiologically acceptable buffers. Preferred non-aqueous carriers include, but are not limited to, mineral oil or neutral oil including, but not limited to, a diglyceride, a triglyceride, a phospholipid, a lipid, an oil and mixtures thereof, wherein the oil contains an appropriate mix of polyunsaturated and saturated fatty acids. Examples include, but are not limited to, soybean oil, canola oil, palm oil, olive oil and myglyol, wherein the number of fatty acid carbons is between 12 and 22 and wherein the fatty acids can be saturated or unsaturated. Optionally,

charged lipid or phospholipid can be suspended in the neutral oil.

In an example, MCC is suspended in DNase-free sterile water and is sonicated at 20% output for 5 minutes (Model W-385 Sonicator, Heat Systems-Ultrasonics Inc). Optionally, the sonicated M-DNA is homogenized by microfluidization at 15,000-30,000 psi for one flow-through (Model M-110Y; Microfluidics, Newton, MA) and is transferred to an autoclaved, capped bottle for storage at 4°C. Optionally, MCC suspensions or M-DNA can be stabilized by the addition of non-ionic or ionic polymers such as polyoxyethylenesorbitan monooleate (Tween) or hyaluronic acid.

In an example, DNase free phosphatidylcholine is added to DNase free triglyceride soybean oil at a ratio of 1 gram of phospholipid to 20 ml of triglyceride and is dissolved by gentle heating at 50°-60°C. Several grams of MCC are added to a dry autoclaved container and the phospholipid-triglyceride solution is added at a concentration of 20 ml per 1 gram of MCC. The suspension is incubated at 20°C for 60 min. and is then mixed with DNase-free PBS in the ratio of 20 ml MCC suspension per liter of DNase-free PBS. The mixture is sonicated at 20% output for 5 minutes (Model W-385 Sonicator, Heat Systems-Ultrasonics Inc.). Optionally, the sonicated MCC mixture is homogenized by microfluidization at 15,000-30,000 psi for one flow-through (Model M-110Y; Microfluidics) and is transferred to an autoclaved capped bottle for storage at 4°C.

The amount of MCC administered per dose, the number of doses and the dose schedule will depend on the type of inflammation, the severity of the inflammation, the location of the inflammation and other clinical factors such as the size, weight and physical condition of the recipient and the route of administration and can be determined by the medical practitioner using standard clinical techniques and without undue experimentation. In addition, *in vitro* assays may optionally be employed to help identify optimal range for MCC administration.

Preferably, the amount of MCC administered is from about

0.00001 to 100 mg/kg per dose, more preferably from about 0.0001 to 50 mg/kg per dose, and most preferably from about 0.001 to 20 mg/kg per dose. Preferably, the M-DNA content of the MCC is between about 0.001 and 90 mg/100 mg dry MCC, more preferably between about 0.01 and 40 mg/100 mg dry MCC, and most preferably between about 0.1 and 30 mg/100 mg dry MCC. Also, it is preferable that the protein content of the MCC be less than about 20 mg/100 mg dry MCC and the extractable M-DNA be at least about 4.5% of the dry weight of MCC.

Routes for administration include, but are not limited to, oral, topical, subcutaneous, transdermal, subdermal, intra-muscular, intra-peritoneal, intra-vesical, intra-articular, intra-arterial, intra-venous, intra-dermal, intra-cranial, intra-inflammation, intra-ocular. intra-pulmonary, intra-spinal, placement within cavities of the body, nasal inhalation, pulmonary inhalation, impression into skin and electrocorporation. Depending on the route of administration, the volume per dose is preferably about 0.0001 ml to about 100 ml per dose, more preferably about 0.001 ml to about 60 ml per dose and most preferably about 0.01 ml to about 40 ml per dose.

The following examples will serve to further illustrate the present invention without, at the same time, however, constituting any limitation thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

EXAMPLE 1

Preparation of MCC

MCC was prepared from *M. phlei* as described in International Patent Application No. PCT/CA98/00744, which is included by reference herein.

Briefly, to prepare MCC, *M. phlei* are grown in liquid medium and harvested. The *M. phlei* are disrupted, and the solid components

of the disrupted *M. phlei* are collected by centrifugal sedimentation. The solid components are modified by deproteinization with DNase-free trypsin and DNase-free pronase, delipidation with DNase free urea and DNase-free phenol and washing with DNase-free water.

All reagents used in the preparation of MCC were selected to enhance conservation of the DNA. Unless stated otherwise, MCC was resuspended in DNase-free water or in a pharmaceutically acceptable DNase-free buffer and emulsified by sonication. MCC did not contain endotoxins as determined using a Limulus amebocyte lysate QCL-1000 kit (BioWhittaker, Walkersville, MD).

EXAMPLE 2

Preparation of BCC from mycobacterial species other than M. phlei

BCC is prepared from mycobacterial species including, but not limited to, M. vaccae, M. chelonei, M. smegmatis, *M. terrae, M. duvalii, M. tubeculosis, M. bovis BCG, M. avium, M. Szulgai, M. scrofulaceum, M. xenopi, M. kansaii, M. gastr, M. fortuitous* and *M. asiaticum* as in Example 1.

EXAMPLE 3

Administration of MCC and induction of inflammation

6.7 mg kg^{-1} MCC in saline (experimental) or saline (control) were administered to female CD-1 mice (Charles River, Saint Constant, Quebec, Canada) intravenously in 0.2 ml; intraperitoneally in 1.0 ml; subcutaneously, into the hind footpad, in 0.05 ml; and, orally, using a feeding needle, in 0.2 ml. Two h later a 1% solution of carrageenan (Sigma-Aldrich, Mississauga, Ontario, Canada) in a final volume of 0.05 ml was injected into the hind footpad of each mouse to induce inflammation. Footpad swelling was quantified by measuring water-displacement at 0, 3, 24, 48, 72 and 96 h after carrageenan injection (Filion et al. British Journal of Pharmacology 122:551-557, 1997).

EXAMPLE 4

Anti-inflammatory effect of MCC

Carrageenan induced inflammation was detected at 3 h, peaked

at 48 h and began to decrease at 72 h. Both intravenous and oral administration of MCC produced a significant reduction in footpad inflammation (volume) within 3 h after carrageenan injection. Maximum reduction of inflammation occurred at 48 h after both intravenous (58% reduction) and oral (57% reduction) administration of MCC and persisted for at least 72 h (Fig. 1 & Fig. 2).

Subcutaneous administration of MCC into the hind footpad, 2 h before carrageenan injection into the same hind footpad, also reduced inflammation. However, this was not evident until 24 h after carrageenan injection. Maximum reduction in inflammation was 40% and occurred at 48 h (Fig. 1 & Fig. 2).

Intraperitoneal administration of MCC provided minimal reduction in inflammation for 48 h and, by 72 h, there was no difference in footpad volume between experimental and control mice (Fig. 1 & Fig. 2).

EXAMPLE 5

IL-10 induction by MCC

The ability of MCC to induce IL-10 and TNF-alpha synthesis was evaluated. IL-10 is an anti-inflammatory cytokine (Isomake et al. Annals of Medicine 29:499-507, 1997). TNF-alpha is a pro-inflammatory cytokine (Shanley et al. Molecular Medicine Today 1:40-45 1995).

Groups of four mice each received 6.7 mg kg^{-1} MCC in 0.2 ml of saline intravenously (experimental), 6.7 mg kg^{-1} or 50 mg kg^{-1} MCC in 1.0 ml of saline intraperitoneally (experimental), 6.7 mg kg^{-1} MCC in 0.2 ml of saline orally (experimental) or saline (control). Blood was obtained from the tail vein of the mice and IL-10 and TNF-alpha in the serum were quantified at 0, 3, 6 and/or 24 h after MCC administration using the appropriate ELISA kit (BioSource, Camarillo, CA).

Mice administered 50 mg kg^{-1} MCC intraperitoneally showed a significant increase in the anti-inflammatory cytokine IL-10, which peaked at 6 h, and showed no significant increase in the pro-inflammatory cytokine TNF-alpha (Fig. 3). Mice administered 6.7 mg

kg^{-1} MCC either intraperitoneally or intravenously showed a minimal increase in IL-10 synthesis at 6 h, whereas mice administered 6.7 mg kg^{-1} MCC orally showed a significant increase in IL-10 synthesis at 6 h (Fig. 4).

EXAMPLE 6

MCC treatment of osteoarthritis

Ten patients with debilitating osteoarthritis were administered MCC intravenously twice per week for four weeks. Eight of the ten patients reported a significant reduction in pain and a significant increase in their ability to perform routine tasks.

EXAMPLE 7

MCC treatment of colitis

Fifteen patients with colitis were divided into three groups. Once each day for sixty days Group 1 patients received saline orally, Group 2 patients received cortisone orally and Group 3 patients receive MCC orally. At the end of the thirty days, Group 1 patients reported no reduction in symptoms. Group 2 patients reported a reduction in colitis symptoms, but complain of cortisone side effects. Group 3 patients reported a reduction in colitis symptoms without any mention of side effects.

It should be understood, of course, that the foregoing relates only to preferred embodiments of the present invention and that numerous modifications or alterations may be made therein without departing from the spirit and the scope of the invention as set forth in the appended claims.

We claim:

1. A method for treating an inflammation in an animal having an inflammation, comprising administering to the animal having the inflammation an effective amount of a composition comprising a mycobacterial deoxyribonucleic acid (B-DNA) preserved and complexed on a mycobacterial cell wall (BCC) and a pharmaceutically acceptable carrier, thereby treating the inflammation in the animal having the inflammation.

2. A method for preventing an inflammation in an animal, comprising administering to the animal an effective amount of a composition comprising a mycobacterial deoxyribonucleic acid (B-DNA) preserved and complexed on a mycobacterial cell wall (BCC) and a pharmaceutically acceptable carrier, thereby preventing the inflammation in the animal.

3. A method according to claim 1 or 2, wherein the B-DNA preserved and complexed on the mycobacterial cell wall (BCC) is *M. phlei*-DNA (M-DNA) preserved and complexed on *M. phlei* cell wall (MCC).

4. A method according to claim 1, 2 or 3 wherein the pharmaceutically acceptable carrier is selected from the group consisting of an aqueous carrier and a non-aqueous carrier.

5. A use of a composition comprising a mycobacterial deoxyribonucleic acid (B-DNA) preserved and complexed on a mycobacterial cell wall (BCC) and a pharmaceutically acceptable carrier for the manufacture of a medicament to treat an inflammation in an animal having an inflammation.

6. A use of a composition comprising a mycobacterial

deoxyribonucleic acid (B-DNA) preserved and complexed on a mycobacterial cell wall (BCC) and a pharmaceutically acceptable carrier for the manufacture of a medicament to prevent an inflammation in an animal.

7. A use according to claim 5 or 6 wherein the B-DNA preserved and complexed on the mycobacterial cell wall (BCC) is *M. phlei*-DNA (M-DNA) preserved and complexed on *M. phlei* cell wall (MCC).

8. A use according to claim 5, 6 or 7 wherein the pharmaceutically acceptable carrier is selected from the group consisting of an aqueous carrier and a non-aqueous carrier.

9. A composition comprising a mycobacterial deoxyribonucleic acid (B-DNA) preserved and complexed on a mycobacterial cell wall (BCC) and a pharmaceutically acceptable carrier.

10. A composition according to claim 9 wherein the B-DNA preserved and complexed on the mycobacterial cell wall (BCC) is *M. phlei*-DNA (M-DNA) preserved and complexed on *M. phlei* cell wall (MCC).

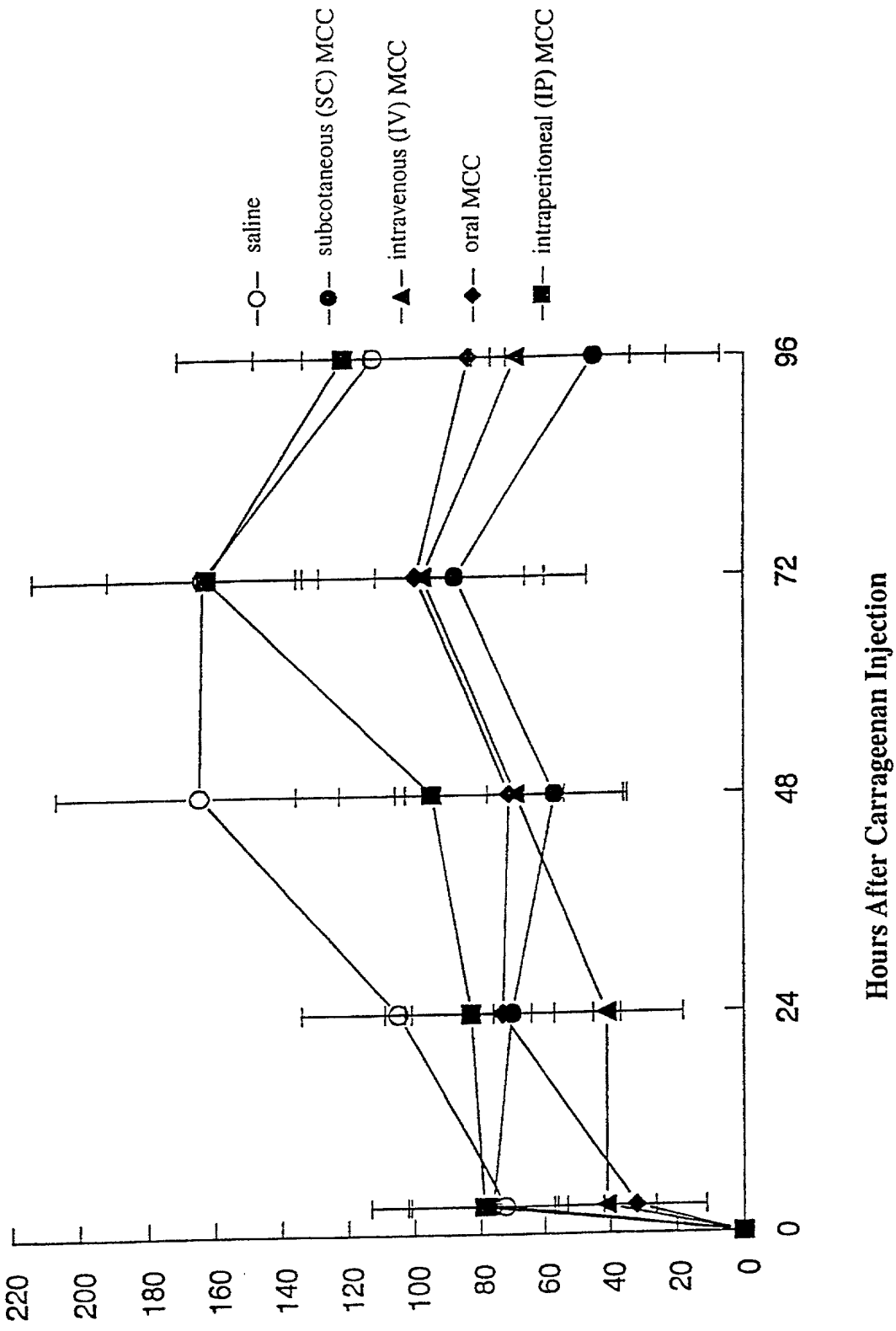
saline
subcotaneous (SC) MCC
intravenous (IV) MCC
oral MCC
intraperitoneal (IP) MCC
Change in Footpad Volume (x 10-2 ml)
220
200
180
160
140
120
100
80
60
40
20
0
0
24
48
72
96
Hours After Carrageenan Injection

Figure 1

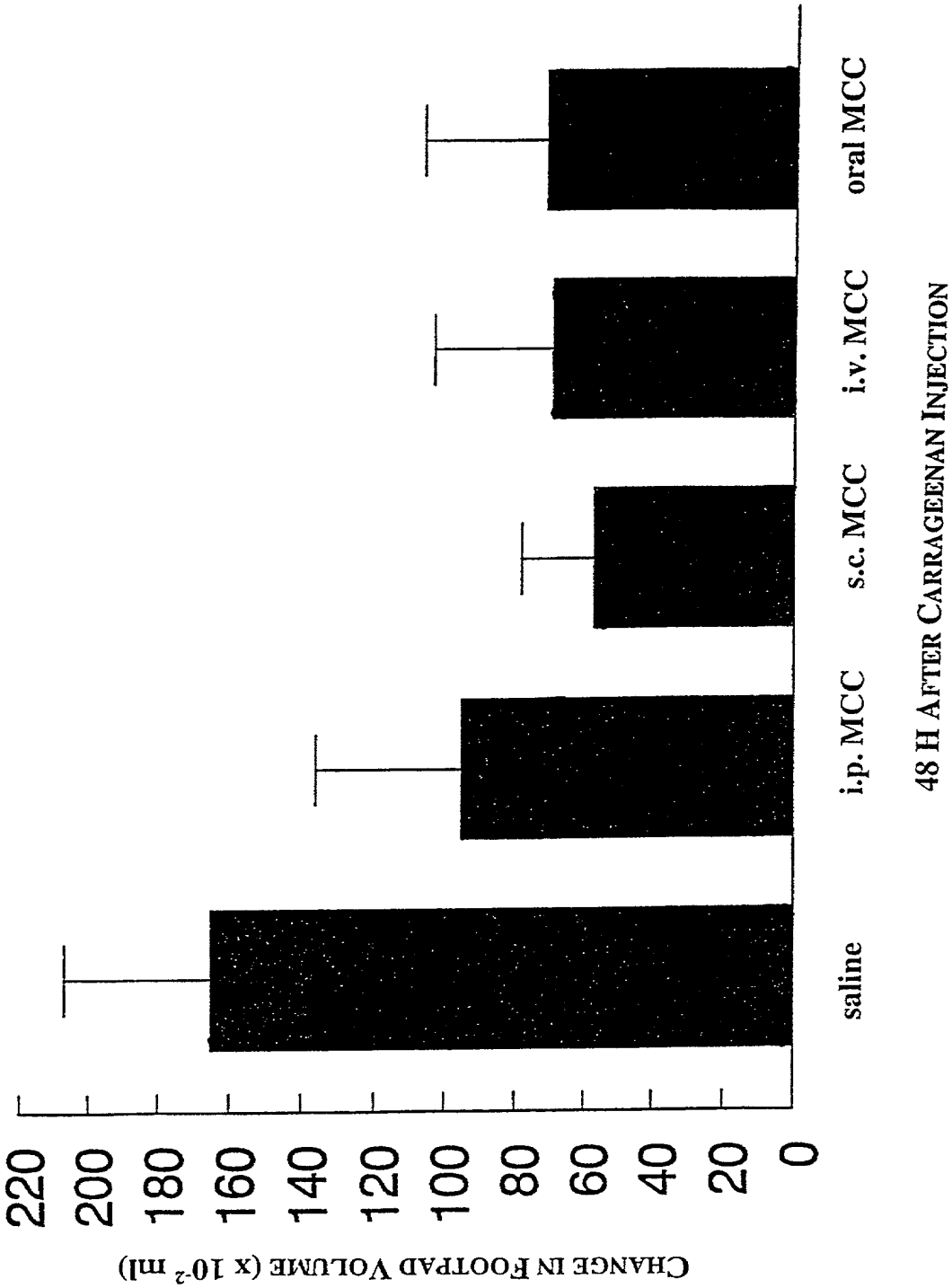
CHANGE IN FOOTPAD VOLUME (x 10-2 ml)
220
200
180
160
140
120
100
80
60
40
20
0
saline
i.p. MCC
s.c. MCC
i.v. MCC
oral MCC
48 H AFTER CARRAGEENAN INJECTION

Figure 2

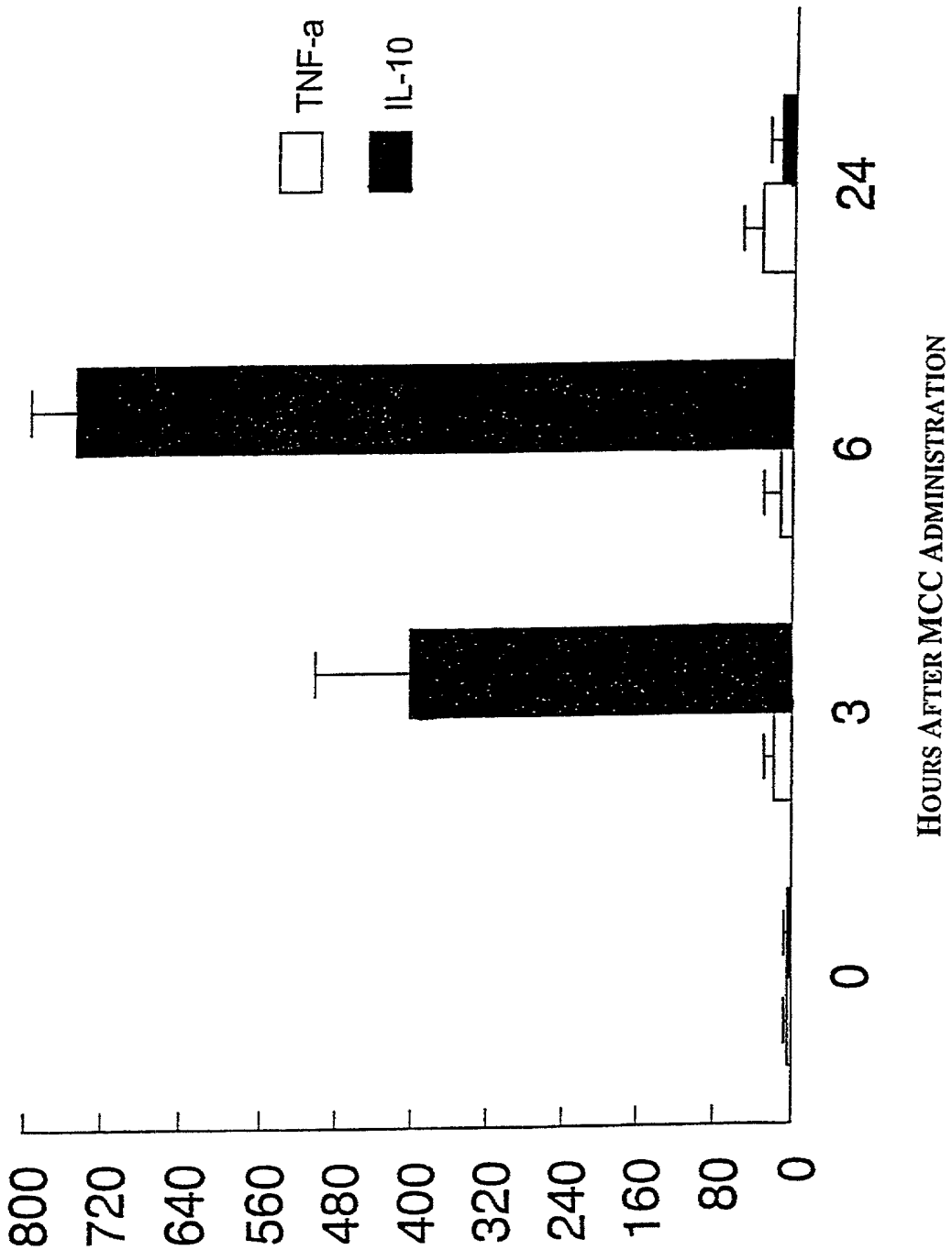
TNF-a
IL-10
800
720
640
560
480
400
320
240
160
80
0
0
3
6
24
HOURS AFTER MCC ADMINISTRATION
CYTOKINE SYNTHESIS (PG/ML)

Figure 3

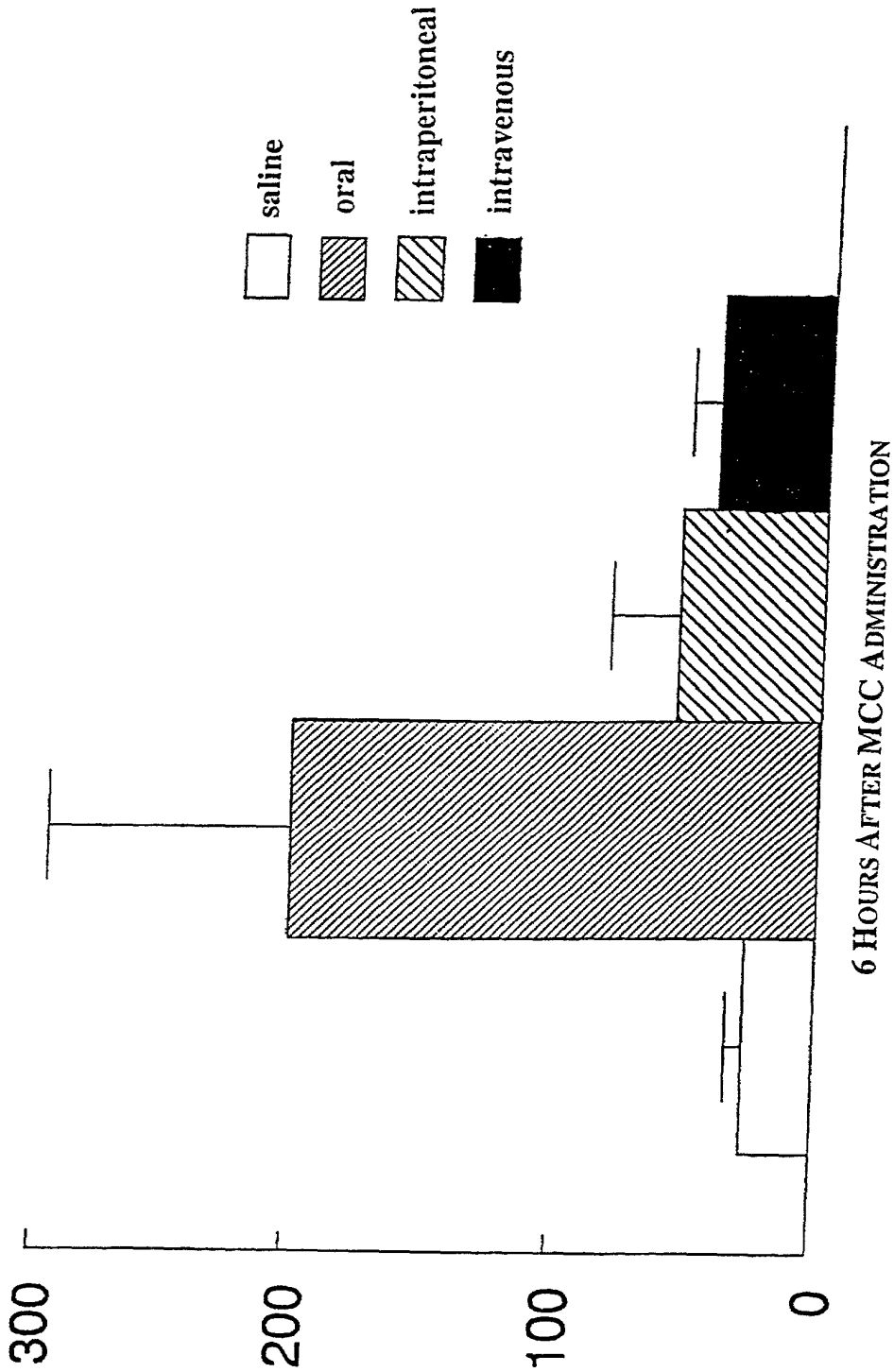
saline
oral
intraperitoneal
intravenous
6 HOURS AFTER MCC ADMINISTRATION
300
200
100
0
IL-10 SYNTHESIS (PG/ML)

Figure 4

DECLARATION AND POWER OF ATTORNEY Attorney's Docket No. **42368-258914(02811-0141US)**

In re Application of: **Nigel C. Phillips and Mario C. Filion**
As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: **Method for the Treatment of Inflammation**, the specification of which:

☐ is attached hereto.
☒ was filed on 6/04/01 as Application No. 09/857,333 (if applicable) and was amended on 6/04/01.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I do not know and do not believe that the same was ever known or used by others in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to the date of this application. I further state that the invention was not in public use or on sale in the United States of America more than one year prior to the date of this application. *I understand that I have a duty of candor and good faith toward the Patent and Trademark Office,* and I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) of the foreign application(s) for patent or inventor's certificate listed below, and have also identified below any foreign application for patent or inventor's certificate disclosing subject matter in common with the above-identified specification and having a filing date before that of the application on which priority is claimed:

Application No.	Country	Filing Date	Priority Claimed Under 35 USC §119
PCT/CA99/01156	PCT	12/03/1999	Yes X No

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

60/110,943	12/04/98		
(Application No.)	(Filing Date)	(Application No.)	(Filing Date)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter disclosed and claimed in the present application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status: patented, pending, abandoned

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statement were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patents issuing thereon.

POWER OF ATTORNEY: The following attorneys are hereby appointed to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: **Customer Number 23370**

PATENT TRADEMARK OFFICE

Direct all correspondence to: **Customer Number 23370**

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23370
PATENT TRADEMARK OFFICE

Direct telephone calls at **404-815-6500**, to **John K. McDonald, Ph.D.**

1-00

Full name of sole or first inventor: **Nigel C. Phillips**	Citizenship: **Canada** CAN
Inventor's signature NC Phillips	Date: 24 August 2001
Residence and Post Office Address: **101 Seigniory Avenue, Pointe-Claire, Quebec H9R 1J6, Canada**	

☒ Additional inventors are being named on separately numbered sheets attached hereto.

2-OP

Full name of second inventor, if any: **Mario C. Filion**	Citizenship: **Canada** CAX
Inventor's signature Mario C. Filion	Date: August 16, 2001
Residence and Post Office Address: **1145 Jean-de Brebeuf St., Laval, Quebec, Canada H4R 2R2**	

Full name of third inventor, if any:	Citizenship:
Inventor's signature	Date:
Residence and Post Office Address:	

Full name of fourth inventor, if any:	Citizenship:
Inventor's signature	Date:
Residence and Post Office Address:	